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(54) Title: DIAGNOSTIC METHODS AND GENE THERAPY USING REAGENTS DERIVED FROM THE HUMAN METASTASIS SUPPRESSOR GENE KAI1

(57) Abstract

The isolation and characterization of a metastasis tumor suppressor gene *KAI1* is disclosed and diagnostic methods and gene therapy approaches utilizing reagents derived from the nucleotide and deduced amino acid sequences of the *KAI1* gene are provided.

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Title of Invention

DIAGNOSTIC METHODS AND GENE THERAPY USING
REAGENTS DERIVED FROM THE HUMAN METASTASIS SUPPRESSOR GENE
KAI1.

5 Field of Invention

This invention is in the field of cancer diagnostics and therapeutics. In particular, this invention relates to detection of alterations of wild-type KAI1 gene sequence, KAI1 mRNA and KAI1 protein useful in determining the presence of malignant cancer in a subject or a genetic predisposition to malignancy in a subject. The invention further relates to the use of gene therapy to restore the wild-type KAI1 gene product.

15 Background of Invention

It has been widely accepted that carcinogenesis is a multistep process involving genetic and epigenetic changes that dysregulate molecular control of cell proliferation and differentiation. The genetic changes can include activation of proto-oncogenes and/or the inactivation of tumor suppressor genes that can initiate tumorigenesis as well as lead to the progression of tumors. For example, the tumor suppressor gene p53 may be involved in late stages of colorectal carcinomas (Baker, S.J. et al., (1989) Science, 244: 217-221) and a putative metastasis suppressor gene, nm23, was found down-regulated in metastatic tumors versus nonmetastatic tumors (Steeg, P.S. et al., (1988) J. Natl. Canc. Inst., 80:200-204). In addition, the activation of ras oncogene and the amplification of N-myc have been associated with progression of human tumors such as breast carcinomas (Liu, E. et al., (1988) Oncogene 3:323-327); and neuroblastomas (Brodeur, G.M. et al., (1984) Science, 224:1121-1124; Schwab, M. et al., (1984) Proc. Natl. Acad. Sci. U.S.A., 81:4940-4944) but they are unlikely to be

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- universal determinants of tumor progression (Nicolson, G.L. Bio Essays, 13:337-342 (1991)).

However despite these advances in understanding the genetic changes underlying carcinogenesis, metastasis, which is the main cause of death for most cancer patients (Rosenberg, S.A., Surgical Treatment of Metastasis Cancer (Lippincott, Philadelphia PA 1987)), remains one of the most important but least understood aspects of cancer (Liotta, L.A. et al. (1991) Cell, 64:327-336; Nicolson, G.L. (1991) BioEssays, 13:337-342 and Steeg, P.S. (1992) Curr. Opin. Oncol., 4:134-141). Accordingly, the isolation of metastasis tumor suppressor genes is of great importance for the diagnosis and therapy of cancers.

Cell fusion studies by Ramshaw et al. ((1983) Int. J. Cancer, 32:471-478) in which hybridization of non-metastatic and metastatic tumor cells produced cell hybrids which are tumorigenic but no longer metastatic demonstrated the existence of metastasis suppressor genes. More recently, Ichikawa et al. (1991) Cancer Res., 51:3788-3792) demonstrated that the metastatic ability of rat prostatic cancer cells was suppressed when fused to non-metastatic cancer cells and that the reexpression of metastasis was associated with the consistent loss of a normal rat chromosome. A subsequent study using micro-cell-mediated chromosome transfer further mapped a putative human metastasis suppressor gene to the 11p11.2-13 region of human chromosome 11. (Ichikawa et al. (1992) Cancer Res., 52:3486-3490) In this study, these researchers demonstrated that a hybrid retaining human chromosome 11cent-p13 showed a suppression of metastasis while hybrids retaining 11cent-p11.2 did not.

In sum, the data presented in the Ichikawa et al. papers suggested that a putative suppressor gene in the p11.2-13 region of human chromosome 11 may play a role in metastasis. However to date, no gene has been identified in this region which is a candidate metastasis

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- suppressor gene. Thus, there is a need in the art to identify such gene(s) in this chromosome region and to determine if any such gene(s) is associated with metastasis.

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Summary of Invention

The present invention relates to methods for detecting alterations of the wild-type *KAI1* gene where detection of such alterations is useful in determining the presence of a malignant cancer in a subject or a genetic predisposition to malignancy in a subject. A first method for detecting alterations of the wild-type *KAI1* gene comprises analyzing the DNA of a subject for mutations of the *KAI1* gene. A second method for detecting alterations of the *KAI1* gene comprises analyzing the RNA of a subject for mutations and altered expression of the mRNA product of the *KAI1* gene.

10 The present invention therefore provides nucleic acid probes for detection of alterations of the wild-type *KAI1* gene.

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The present invention further provides a diagnostic kit containing purified and isolated nucleic acid sequences useful as PCR primers in analyzing RNA or DNA of a subject for alterations of the wild-type *KAI1* gene. These PCR primers can also be used to determine the nucleotide sequence of *KAI1* alleles.

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A third method for detecting alterations of the wild-type *KAI1* gene comprises analyzing protein of a subject for alterations in the expression of *KAI1* protein.

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The invention therefore relates to antibodies to the *KAI1* protein and to a diagnostic kit containing antibodies to *KAI1* protein useful for detecting alterations in *KAI1* protein expression in a subject.

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The present invention further provides a method for supplying the wild-type *KAI1* gene to a cell having altered expression of the *KAI1* protein, the method

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- comprising: introducing a wild-type *KAI1* gene into a cell having altered expression of *KAI1* protein such that the wild-type gene is expressed in the cell.

Description of Figures

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Figure 1 shows the results of a Northern blot of mRNA from normal tissue (human prostate) and from both metastatic (AT6.1, AT6.1-11-2 and AT6.1-11-3) and non-metastatic (AT6.1-11-1*) tumor cells. 2 μ g of poly A⁺ RNA per sample was loaded in each lane and the blots were hybridized sequentially with *KAI1* cDNA and rat β -actin probes. The asterisk (*) identifies the hybrid AT6.1-11-1 that contained the 11pcen-p13 region and was suppressed in metastatic ability.

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25 Figure 3 shows the nucleotide (upper line) and
 deduced amino acid (lower line) sequences of the *KAI1* cDNA
 where the abbreviations for the amino acid residues are:
 A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I,
 Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R,
 30 Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr. The four
 putative transmembrane domains are noted by a dotted
 underline and the potential N-linked glycosylation sites
 are doubly underlined.

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- metastatic prostate cancers. The blot was hybridized sequentially with *KAI1* and human β -actin probes.

Figure 5 shows the results of Northern blot analysis of 2 μ g of poly A⁺ RNA prepared from the various human tissues indicated at the top of Figure 5. The blot was hybridized sequentially with *KAI1* and human β -actin probes.

Figure 6 shows the results of a "zoo" blot of EcoRI-digested genomic DNA of the various species indicated at the top of Figure 6. The blot was hybridized with *KAI1* probe.

Detailed Description of Invention

The present invention relates to the cloning and characterization of a metastasis suppressor gene on human chromosome 11. The nucleotide and deduced amino acid sequences of this gene, designated *KAI1* herein, are shown in Figure 3. The nucleotide sequence shown in Figure 3 was cloned from a metastasis suppressed cell hybrid clone AT6.1-11-1* and represents the wild-type *KAI1* sequence.

A search of the *KAI1* cDNA sequence in GenBank and EMBL databases revealed that the *KAI1* cDNA sequence is identical to three cDNA clones from human lymphocytes, designated C33, R2 and IA4 by different laboratories (Imai, T. et al. (1992) *J. Immunol.*, 149, 2879-2886 (1992); Fukudome, K. et al. (1992) *J. Virol.*, 66, 1394-1401 (1992); Gaugitsch, H. W., et al. (1991) *Eur. J. Immunol.*, 21, 377-383 (1991); Gil, M. L. et al. (1992) *J. Immunol.*, 148, 2826-33 (1992)). C33 is associated with the inhibition of virus-induced syncytium formation (Imai, T. et al. (1992); Fukudome, K. et al. (1992)); R2 is strongly up-regulated in mitogen-activated human T cells (Gaugitsch, H. W., et al. (1991)), and IA4 is highly expressed in several B lymphocyte lines (Gil, M. L. et al. (1992)). However, none of these three clones were suggested to function in metastasis and the function of

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- the protein encoded by these clones was not known prior to the present invention.

The present invention further relates to association of alterations of the wild-type *KAI1* gene with metastasis. Accordingly, the present invention relates to 5 methods for detecting alterations of the wild-type *KAI1* gene in a subject where such methods can provide diagnostic and prognostic information. For example, since loss of expression of the *KAI1* gene has been observed in metastatic prostate tumors, these are tumors in which *KAI1* has a role in metastasis. Thus, detection of alterations 10 of the wild-type *KAI1* gene in a subject may effect the course of treatment chosen by a clinician. In addition, since *KAI1* is expressed in all tissues tested including 15 spleen, thymus, prostate, testes, ovary, small intestine, colon, blood leukocyte, heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas, alterations of the wild-type *KAI1* gene may contribute to metastasis in these tissues.

It is further understood by those of ordinary 20 skill in the art that the methods of the present invention are applicable to any tumor in which alterations of wild-type *KAI1* occur. Moreover, the methods of detection disclosed in the present invention can be used prenatally 25 to screen a fetus or presymptomatically to screen a subject at risk of having cancer based on his/her family history. For purposes of the present invention, subject means a mammal.

According to the diagnostic methods of the 30 present invention, alterations of the wild-type *KAI1* gene are detected. "Alterations of the wild-type *KAI1* gene" as used throughout the specification and claims encompasses mutations of the wild-type *KAI1* gene where such mutations include deletions, inversions, insertions, transversions or point mutations of the wild-type *KAI1* gene. It is believed that many mutations found in tumor tissues will 35

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- be those leading to decreased expression of *KAI1* protein. However, mutations leading to non-functional gene products can also lead to malignancy. It is further understood that point mutations can occur in regulatory regions (e.g. promoter) or can disrupt proper RNA processing thus leading to loss of expression of the *KAI1* gene products respectively.

5 "Alterations of the wild-type *KAI1* gene" as used throughout the specification and claims can also be detected on the basis of altered expression of the wild-type *KAI1*-specific mRNA and *KAI1* protein. The altered expression of these *KAI1* gene products may be detected as a loss or reduction in the levels of *KAI1* mRNA and protein relative to wild-type levels. Alternatively, the altered expression of *KAI1* protein can encompass a loss of function of the *KAI1* protein. Those of ordinary skill in 10 the art would therefore understand that altered expression of the *KAI1* gene products may be caused by a variety of events, including but not limited to, mutations of the wild-type *KAI1* gene, changes in the posttranslational 15 modification of the *KAI1* protein (e.g. glycosylation) or 20 loss of a trans-acting factor necessary for transcription of the *KAI1* gene.

25 Provided with the *KAI1* cDNA and deduced amino acid sequences shown in Figure 3, design of particular probes useful in detecting alterations of the wild-type *KAI1* gene is well within the skill of the ordinary artisan.

30 In one embodiment of the invention, the method for detecting alterations of the *KAI1* gene comprises analyzing the DNA of a subject for mutations of the wild-type *KAI1* gene. For analysis of DNA, a biological specimen is obtained from the subject. Examples of 35 biological specimens that can be obtained for use in the present methods include, but are not limited to, tissue biopsies, whole blood, lymphocytes and tumors. Means for

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- enriching a tissue preparation for tumor cells are known in the art. For example, the tissue may be isolated from paraffin or cryostat sections. Cancer cells may also be separated from normal cells by flow cytometry and other techniques well known in the art. Alternatively, primary cell cultures can be established from tumor biopsies using methods known to those of ordinary skill in the art.

The DNA isolated from the biological specimen can be analyzed for mutations of the wild-type *KAI1* gene by a variety of methods including, but not limited to, 5 Southern blotting after digestion with the appropriate restriction enzymes (restriction fragment length polymorphism, RFLP) (Botstein, D. (1980) Amer. J. Hum. Genet., 69:201-205, denaturing gradient electrophoresis technique (Myers, R.M., (1985) Nature, 313:495-498), 10 oligonucleotide hybridization (Conner, R. et al., (1984) EMBO J., 3:13321-1326), RNase digestion of a duplex 15 between a probe RNA and the target DNA (Winter, E. et al., (1985) Proc. Natl. Acad. Sci. U.S.A., 82:7575-7579), polymerase chain reaction (PCR) (Saiki, P.K. et al., 20 (1988) Science, 239:487-491; U.S. Patents 4,683,195 and 4,683,202), ligase chain reaction (LCR) (European Patent Application Nos. 0,320,308 and 0,439,182), and PCR-single stranded conformation analysis (PCR-SSCP) (Orita, M. et al. (1989) Genomics, 5:874-879; Dean, M. et al. (1990) Cell, 61:863-871).

In one preferred embodiment, Southern blot analysis can be used to examine DNA isolated from a subject for gross rearrangement of the *KAI1* gene. The DNA to be analyzed via Southern analysis is digested with one or more restriction enzymes. Following restriction 30 digestion, resultant DNA fragments are separated by gel electrophoresis and the fragments are detected by hybridization with a labelled nucleic acid probe (Southern, E.M. (1975) J. Mol. Biol., 98:503-517).

The nucleic acid sequence used as a probe in 35

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• Southern analysis can be labeled in single-stranded or double-stranded form. Labelling of the nucleic acid sequence can be carried out by techniques known to one skilled in the art. Such labelling techniques can include radiolabels and enzymes (Sambrook, J. et al. (1989) in "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Press, Plainview, New York). In addition, there are known non-radioactive techniques for signal amplification including methods for attaching chemical moieties to pyrimidine and purine rings (Dale, R.N.K. et al. (1973) Proc. Natl. Acad. Sci., 70:2238-2242; Heck, R.F. 1968) S. Am. Chem. Soc., 90:5518-5523), methods which allow detection by chemiluminescence (Barton, S.K. et al. (1992) J. Am. Chem. Soc., 114:8736-8740) and methods utilizing biotinylated nucleic acid probes (Johnson, T. K. et al. (1983) Anal. Biochem., 133:126-131; Erickson, P.F. et al. (1982) J. of Immunology Methods, 51:241-249; Matthaei, F.S. et al. (1986) Anal. Biochem., 157:123-128) and methods which allow detection by fluorescence using commercially available products. Each of the nucleic acid sequences used as a probe in Southern analysis is derived from the wild-type KAI1 gene. Preferred probes are derived from having the cDNA sequence shown in Figure 3.

Once the separated DNA fragments are hybridized to the labelled nucleic acid probes, the restriction digest pattern can be visualized by autoradiography and compared with the restriction digest pattern of the wild-type KAI1 gene. The presence or absence of a restriction fragment length polymorphism (RFLP) in the restriction pattern of the subject's DNA relative to the wild-type restriction pattern indicates an alteration of the wild-type KAI1 gene.

In another preferred embodiment, genomic DNA may be analyzed for mutations in the wild-type KAI1 gene via PCR-SSCP. In this method, each of the pair of primers selected for use in PCR are designed to hybridize with

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- sequences in the wild-type *KAI1* gene to permit amplification and subsequent detection of mutations in the denatured amplification product via non-denaturing polyacrylamide gel electrophoresis. In one embodiment, primer pairs are derived from the *KAI1* cDNA sequence shown 5 in Figure 3.

In another embodiment, primer pairs useful in the analysis of genomic DNA mutations of the wild-type *KAI1* gene may be derived from intronic sequences which border the 5' and 3' ends of a given exon of the *KAI1* 10 gene. Examples of primer pairs permitting specific amplification of specific exons of the *KAI1* gene include:

SEQ ID NO:1: AGAAGATCAAGTTGAAGAGG

SEQ ID NO:2: GGGACCTCATTCTCTAGCTG

SEQ ID NO:3: ATGAAACTGCTCTTGTGCGG

15 SEQ ID NO:4: TCAGCTCTGGCTCCCCATT

SEQ ID NO:5: TGGGCACGGGTTTCAGGAAAT

SEQ ID NO:6: TGCAGAGAGCCCCAAATGCA

SEQ ID NO:7: AGGGTGAGCCGTGAGCACAA

SEQ ID NO:8: TGCTGAGAGTACCCAGATGC

20 SEQ ID NO:9: GATGGCCACACCCACGGCC

SEQ ID NO:10: TGCATGGAGAAGGTGCAGGC

SEQ ID NO:11: CCTCTTGCCACCCCTGACTGA

SEQ ID NO:12: TTCACACCATTCTCCTGCCT

where SEQ ID NOS:1 and 2 bound exon 3; SEQ ID NOS:3 and 4 bound exon 4; SEQ ID NOS: 5 and 6 bound exon 6; SEQ ID NOS:7 and 8 bound exon 7; SEQ ID NOS:9 and 10 bound exon 8; and SEQ ID NOS:11 and 12 bound exon 9.

30 Each primer of a pair is a single-stranded oligonucleotide of about 15 to about 50 base pairs in length which is complementary to a sequence at the 3' end of one of the strands of a double-stranded target sequence. Optimization of the amplification reaction to obtain sufficiently specific hybridization to the *KAI1* gene sequence is well within the skill in the art and is 35 preferably achieved by adjusting the annealing

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• temperature. In yet another embodiment, RNA may be analyzed for mutations in the *KAI1* gene by RT-PCR-SSCP. In this method, single stranded cDNA is prepared from either total RNA or polyA⁺-enriched RNA using reverse transcriptase. In this method, each of the pairs of primers selected for use in PCR of the resultant single-stranded cDNA are designed to hybridize with sequences in the *KAI1* cDNA which are an appropriate distance apart (at least about 100-300 nucleotides) in the gene to permit amplification and subsequent detection of mutations in the denatured amplification product via non-denaturing polyacrylamide gel electrophoresis. Such primer pairs can be derived from the *KAI1* cDNA sequence set forth in Figure 3. Each pair comprises two such primers, complementary to sequences on each strand separated by generally about 100 to about 300 base pairs.

The primers of this invention can be synthesized using any of the known methods of oligonucleotide synthesis (e.g., the phosphodiester method of Agarwal et al. (1972) Agnew. Chem. Int. Ed. Engl., 11:451, the phosphotriester method of Hsiung et al. (1979). Nucleic Acids Res., 6:1371, or the automated diethylphosphoramidite method of Beuacage et al. (1981). Tetrahedron Letters, 22:1859-1862), or they can be isolated fragments of naturally occurring or cloned DNA. In addition, those skilled in the art would be aware that oligonucleotides can be synthesized by automated instruments sold by a variety of manufacturers or can be commercially custom ordered and prepared. In one embodiment, the primers can be derivatized to include a detectable label suitable for detecting and/or identifying the primer extension products (e.g., biotin, avidin, or radiolabelled dNTP's), or with a substance which aids in the isolation of the products of amplification (e.g. biotin or avidin).

The present invention therefore provides a

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- diagnostic kit for detecting mutations of the KAI1 gene. This diagnostic kit comprises purified and isolated nucleic acid sequences useful as hybridization probes or as PCR primers in analyzing DNA or RNA for alterations of the wild-type KAI1 gene.

5 In an alternative embodiment, nucleic acid probes can be selected to hybridize to mutant alleles of the KAI1 gene. These allele-specific probes are useful to detect similar mutations in other subjects on the basis of hybridization rather than mismatches. Where such nucleic acid probes are primer pairs which hybridize to mutations in the KAI1 gene sequence, these primer pairs can be used to amplify specific mutant gene sequences present in a 10 biological sample via PCR.

15 The amplification products of PCR can be detected either directly or indirectly. Direct detection of the amplification products is carried out via labelling of primer pairs. Labels suitable for labelling the primers of the present invention are known to one skilled in the art and include radioactive labels, biotin, avidin, 20 enzymes and fluorescent molecules. The desired labels can be incorporated into the primers prior to performing the amplification reaction. A preferred labelling procedure utilizes radiolabelled ATP and T4 polynucleotide kinase (Sambrook, J. et al. (1989) in "Molecular Cloning, A 25 Laboratory Manual", Cold Spring Harbor Press, Plainview, NY). Alternatively, the desired label can be incorporated into the primer extension products during the amplification reaction in the form of one or more labelled dNTPs. In the present invention, the labelled amplified 30 PCR products can be analyzed for mutations of the KAI1 gene via separating the PCR products by non-denaturing polyacrylamide gel electrophoresis, denaturing polyacrylamide gel electrophoresis (PCR-SSCP) or via direct sequencing of the PCR-products.

35 In yet another embodiment, unlabelled

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- amplification products can be analyzed for mutations in the *KAI1* disease gene via hybridization with nucleic acid probes radioactively labelled or, labelled with biotin, in Southern blots or dot blots. Nucleic acid probes useful in this embodiment are those described earlier for Southern analysis. In a further embodiment, detection of point mutations may be accomplished by molecular cloning of the allele present in the tumor tissue using the cDNA sequence set forth in Figure 3 and sequencing that allele using techniques well known in the art.

A second method for detecting alterations of the wild-type *KAI1* gene comprises analyzing the RNA of a subject for mutations and altered expression of *KAI1*-specific mRNA.

For the analysis of RNA by this method, RNA can be isolated from, for example, a tumor biopsy sample obtained from said subject where said tumors include, but are not limited to, prostate tumors.

The RNA to be analyzed can be isolated from blood or tumor biopsy samples as whole cell RNA or as poly(A)⁺ RNA. Whole cell RNA can be isolated by methods known to those skilled in the art. Such methods include extraction of RNA by differential precipitation (Birnboim, H.C. (1988) Nucleic Acids Res., 16:1487-1497), extraction of RNA by organic solvents (Chomczynski, P. et al. (1987) Anal. Biochem., 162:156-159) and extraction of RNA with strong denaturants (Chirgwin, J.M. et al. (1979) Biochemistry, 18:5294-5299). Poly(A)⁺ RNA can be selected from whole cell RNA by affinity chromatography on oligo-d(T) columns (Aviv, H. et al. (1972) Proc. Natl. Acad. Sci., 69:1408-1412).

The methods for analyzing RNA for mutations and altered expression of *KAI1*-specific mRNA include Northern blotting (Alwine, J.C. et al. (1977) Proc. Natl. Acad. Sci., 74:5350-5354), dot and slot hybridization (Kafatos, F.C. et al. (1979) Nucleic Acids Res., 7:1541-1522),

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- filter hybridization (Hollander, M.C. et al. (1990) *Biotechniques*; 9:174-179), S₁ analysis (Sharp, P.A. et al., (1980) *Meth. Enzymol.*, 65:750-768), RNase protection (Sambrook, J. et al. (1989) in "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Press, Plainview, NY), reverse-transcription polymerase chain reaction (RT-PCR) (Watson, J.D. et al. (1992) in "Recombinant DNA" Second Edition, W.H. Freeman and Company, New York) and RT-PCR-SSCP.

5 Where expression of *KAI1* mRNA is measured, diminished *KAI1* mRNA expression is indicative of alteration of the wild-type *KAI1* gene. One preferred method for measuring alterations in the level of *KAI1*-specific mRNA expression is Northern blotting where the nucleic acid sequence used as a probe for detecting *KAI1*-specific mRNA expression is complementary to all or part 10 of the *KAI1* cDNA sequence shown in Figure 3.

15 A second preferred method for measuring, alterations in the level of *KAI1*-specific mRNA expression is detection of *KAI1* mRNA expression via hybridization of 20 a nucleic acid probe derived from *KAI1* cDNA sequence to RT-PCR products generated from RNA isolated from a biological sample.

25 A third method for detecting alterations of the wild-type *KAI1* gene comprises analyzing the protein of a subject for alteration of wild-type *KAI1* protein. In one embodiment, alteration of wild-type *KAI1* protein encompasses a loss or reduction in the level of expression 30 of *KAI1* protein in a biological sample.

35 Examples of immunoassays useful in determining the level of expression of *KAI1* protein include, but are not limited to, immunoprecipitation, radioimmunoassay, Western blot assay, immunofluorescent assay, enzyme immunoassay, chemiluminescent assay, immunohistochemical assay and enzyme-linked immunosorbent assay (ELISA). In addition, the above immunoassays may be used in

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combination such as immunoprecipitation followed by Western blot. The above methods are described in Principles and Practice of Immunoassay, Price and Newman, eds., Stochton Press, 1991. Such assays may be a direct, indirect, competitive or noncompetitive immunoassay as described in the art (Oelbrick, N. (1984) J. Clin. Chem. Clin. Biochem., 22:895-904). The protein to be analyzed by such methods may be obtained from biological samples such as tumor biopsies and the protein can be obtained as a crude lysate or it can be further purified by methods known to those of ordinary skill in the art including immunoaffinity chromatography using antibodies to the KAI1 protein (Sambrook, J. et al (1989) in "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Press, Plainview, NY). Alternatively, levels of KAI1 protein may be detected by immunohistochemistry of fixed or frozen tumor sections.

For detection of KAI1 protein by immunoassay, the present invention provides anti-KAI1 antibodies where such antibodies may be polyclonal or monoclonal. If polyclonal antibodies are desired, serum containing polyclonal antibodies to KAI1 protein can be used or the polyclonal antibodies can be purified from other antigens present in the serum by immunoaffinity chromatography. Alternatively, monoclonal antibodies directed against KAI1 can readily be produced by one of ordinary skilled in the art. Methods of producing monoclonal or polyclonal antibodies are known to one of ordinary skilled in the art (Goding, J.W. (1983) monoclonal antibodies: Principles and Practice, Plodermic Press, Inc., NY, NY, pp. 56-97; Hurn, B.A.L. et al. (1980) Meth. Enzymol., 70:104-141).

Suitable immunogens which may be used to produce the polyclonal or monoclonal antibodies of the present invention include cell lysate prepared from cells transfected with a recombinant KAI1 protein, partially or substantially purified recombinant or native KAI1 protein,

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- or peptides derived from the *KAI1* amino acid sequence shown in Figure 3. When purification of the recombinant or native *KAI1* protein is desired, it can be accomplished by standard protein purification procedures known in the art which may include differential precipitation, 5 molecular sieve chromatography, ion-exchange chromatography, isoelectric focusing, gel electrophoresis, affinity, and immunoaffinity chromatography and the like. In the case of immunoaffinity chromatography, the recombinant protein may be purified by passage through a 10 column containing a resin which has bound thereto antibodies specific for the *KAI1* protein.

In a preferred embodiment, the immunogen is a recombinantly produced *KAI1* protein or fragments thereof. Production of recombinant *KAI1* protein or a fragment thereof may be directed by a natural or synthetic nucleic acid sequence inserted into a suitable expression vector. 15 A preferred nucleic acid sequence is the *KAI1* cDNA sequence shown in Figure 3. In one embodiment, restriction digest fragments containing the full-length cDNA or fragments thereof containing a coding sequence for 20 *KAI1* can be inserted into a suitable expression vector. By suitable expression vector is meant a vector that can function in eukaryotic or prokaryotic cells and is capable of carrying and expressing a nucleic acid sequence 25 encoding the *KAI1* protein or a fragment thereof. Such vectors and their use in producing recombinant proteins are known to those of ordinary skill in the art (Sambrook, J. et al. (1989) in "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Press, Plainview, NY).

30 The immunogen of the present invention can be used in a suitable diluent such as saline or water, or in complete or incomplete adjuvants. Further, the immunogen may or may not be bound to a carrier to make the protein immunogenic. Examples of such carrier molecules include 35 but are not limited to bovine serum albumin (BSA), keyhole

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- limpet hemocyanin (KLH), tetanus toxoid, and the like. The immunogen can be administered by any route appropriate for antibody production such as intravenous, intraperitoneal, intramuscular, subcutaneous, and the like. The immunogen may be administered once or at periodic intervals until a significant titer of anti-KAI1 antibody is produced. The antibody may be detected in the serum using an immunoassay.

5 The antibodies or antigen binding fragments may also be produced by genetic engineering. The technology for expression of both heavy and light chain genes in E. coli is the subject of PCT patent applications; publication number WO 901443, WO 901443, and WO 9014424 and in Huse et al. (1989) Science, 246:1275-1281.

10 Alternatively, anti-KAI1 antibodies can be induced by administering anti-idiotype antibodies as immunogens. Conveniently, a purified anti-KAI1 antibody preparation prepared as described above is used to induce anti-idiotype antibody in a host animal. The composition is administered to the host animal in a suitable diluent. Following administration, usually repeated administration, 15 the host produces anti-idiotype antibody. To eliminate an immunogenic response to the Fc region, antibodies produced by the same species as the host animal can be used or the Fc region of the administered antibodies can be removed. Following induction of anti-idiotype antibody in the host 20 animal, serum or plasma is removed to provide an antibody composition. The composition can be purified as described above for anti-KAI1 antibodies, or by affinity chromatography using anti-KAI1 antibodies bound to the 25 affinity matrix.

30 In an alternative embodiment, the antibodies of the present invention can be used in situ to detect KAI1 protein in cells or tissues. In one embodiment, the antibodies are used in direct or indirect immunofluorescence. In the direct method, anti-KAI1 35

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- antibody labelled with a fluorescent reagent such as fluorescein isothiocyanate, rhodamine B isothiocyanate and the like is reacted directly with the KAI1 present in cells or tissues. In the indirect method, unlabelled anti-KAI1 antibody is reacted with the KAI1 protein present in cells or tissue. The unlabelled anti-KAI1 antibody is then reacted with a labelled second antibody. The second antibody can be labelled with a fluorescent tag as described above. The fluorescently labelled cells or tissues can then be detected using techniques known to one skilled in the art such as a fluorescence-activated cell sorter, light microscopy using a fluorescent light lamp and the like. Alternatively, KAI1 protein can be detected *in situ* via the use of radiolabelled anti-KAI1 antibody or via the use of an unlabelled anti-KAI1 antibody followed by a radiolabelled second antibody reactive to the anti-KAI1 antibody.

The antibodies of the present invention may also be used to immunoprecipitate the KAI1 protein from a mixture of proteins. The use of immunoprecipitation as a sensitive and specific technique to detect and quantitate target antigen in mixtures of proteins is well known to those of ordinary skill in the art (see Molecular Cloning, A Laboratory Manual, 2d Edition, Maniatis, T. et al. eds. (1989) Cold Spring Harbor Press).

The antibodies of the present invention may also be affixed to solid supports for use in the isolation of KAI1 protein by immunoaffinity chromatography. Techniques for immunoaffinity chromatography are known in the art (Harlow, E. and Lane, D. (1888) "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) including techniques for affixing antibodies to solid supports so that they retain their immunoselective activity; the techniques used may be those in which the antibodies are adsorbed to the support as well as those in which the antibodies are covalently

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- linked to the support. Generally, the techniques are similar to those used in covalent linking of antigen to a solid support; however, spacer groups may be included in the bifunctional coupling agents so that the antigen binding site of the antibody remains accessible.

5 The above described antibodies and antigen binding fragments thereof may be supplied in a diagnostic kit useful for the detection of alterations in the expression of *KAI1* protein.

10 In a second embodiment, alteration of wild-type *KAI1* protein encompasses loss of function of the *KAI1* protein. The present method therefore includes assays useful in determining the functional status of the *KAI* protein. For example, since an association between processing of N-linked oligosaccharides and metastatic 15 phenotype has been well-documented (Hakomori, S.-I. (1989) Advanc. Cancer Res., 52:257-331; Dennis, J. W., et al. (1987) Science, 236:582-585; Ishikawa, M. et al. (1988) Cancer Res., 48:665-670) it is believed that glycosylation of the *KAI1* protein is required for the protein to 20 function as a metastasis suppressor. Thus, detection of a loss of function of *KAI1* protein as evidenced by an absence of glycosylation is indicative of the presence of metastatic cancer in a subject.

25 The present invention also relates to a gene therapy method in which an expression vector containing a nucleic acid sequence representing the wild-type *KAI1* gene is administered to a subject having a mutation of the *KAI1* gene. A nucleic acid sequence representing wild-type *KAI1* gene is that shown in Figure 3. Such nucleic acid 30 sequence may be inserted into a suitable expression vector by methods known to those of ordinary skill in the art. Expression vectors suitable for producing high efficiency gene transfer in vivo include retroviral, adenoviral and vaccinia viral vectors.

35 Expression vectors containing a nucleic acid

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- sequence representing wild-type KAI1 gene can be administered intravenously, intramuscularly, subcutaneously, intraperitoneally or orally. A preferred route of administration is intraperitoneally.

Any articles or patents referenced herein are incorporated by reference. The following examples are presented to illustrate various aspects of the invention but are in no way intended to limit the scope thereof.

Examples

Materials and Methods

Cell lines: AT6.1 is a highly metastatic Dunning rat prostatic cancer cell line. AT6.1-11 clones are microcell hybrids that have a portion of human chromosomes 11 as the sole human genetic materials in AT6.1 cells. Microcell hybrid AT6.1-11-1* contains a fragment of human chromosome 11, cen-p13, from the centromere to region p13, and was suppressed for metastatic ability. Microcell hybrids AT6.1-11-2 and -3 have smaller fragments of human chromosome 11 from the centromere to p11.2 and were not suppressed for metastatic ability. The characteristics and growth condition for these cell lines have been previously described in detail (Ichikawa et al, (1992) Cancer Res., 52:3486-3490). A9-11neo is a mouse A9 cell line containing human chromosome 11pter-q23 (Koi et al, (1989) Mol Carcinog., 2:12-21).

Isolation and sequencing of KAI1 cDNA clone:

Poly (A)⁺ RNA was isolated from exponentially growing AT6.1-11-1* cells, using a FastTrack mRNA isolation kit (Invitrogen, San Diego, CA). Oligo (dT) was used to prime the first strand cDNA synthesis from 5 µg of poly (A)⁺ RNA. Double-stranded cDNA was cloned into plasmid pSPORT 1 vector by procedures recommended by the vendor (GIBCO BRL, Grand Island, NY). Human Alu sequence primer Alu 559

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◦ (Nelson, D.L. et al (189) Proc. Natl. Acad. Sci U.S.A. 86:6686-6690.) was used to amplify genomic DNA from suppressed hybrid AT6.1-11-1* and the nonsuppressed clone AT6.1-11-2 by PCR. The multiple Alu-PCR fragments of AT6.1-11-1* were cloned into a T-tailed vector pCR1000 (Invitrogen, San Diego, CA). Individual clones corresponding to each fragment of Alu-PCR products were isolated after comparing the size of these Alu-PCR products to molecular weight markers in a agarose gel stained with ethidium bromide. Eleven fragments unique to AT6.1-11-1* were labeled by random priming (GIBCO BRL, Bethesda, MD) and used to screen 5×10^4 recombinants of the cDNA library under stringent wash conditions (65°C in $0.1 \times \text{SSC} + 0.1\%$ SDS for 30 min.). Five independent clones were obtained and their inserts were sequenced using the 15 Sequenase kit (US Biochemical, Cleveland, OH). DNA sequences were analyzed with the GCG package (version 7.3, 1993, Madison, WI).

RNA analysis: Cytoplasmic RNA from AT6.1, AT6.1-11-1*, -2 and -3 were prepared from exponentially growing cells, using FastTrack mRNA isolation kit (Invitrogen, San Diego, CA). Other poly (A)⁺RNA and human multiple tissue Northern blots were purchased from Clontech (Palo Alto, CA). 2 μg of poly (A)⁺RNA was denatured with formamide and fractionated on a 1.2% agarose gel in formaldehyde buffer. The RNA was then transferred onto nylon membrane, baked in an oven at 80°C for 90 min, and then hybridized with a labeled probe in QuickHyb hybridization solution (Stratagene, La Jolla, CA) at 68°C for 1.5 hours and washed at 68°C for 30 minutes in 30 $0.1 \times \text{SSC}$, 0.1% SDS and autoradiographed.

DNA analysis: 15 μg of genomic DNA was digested with BamHI and separated on a 1.2% agarose gel. Following denaturation and neutralization, the DNA in the gel was transferred onto nylon membrane. The "zoo" blot containing EcoRI-digested genomic DNA from human, rat, 35

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- mouse, dog, cow, rabbit, chicken and yeast (Zoo-blot) was purchased from Clontech (Palo Alto, CA). The Southern blots were further hybridized and washed under the same conditions as described above for the Northern blots.

5 PCR: All PCRs in this study were carried out in 50 μ l with 25 pmol of each primer, 10 mM Tris.HCl, 500 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 250 mM of each dNTP and .25 units of Taq DNA polymerase (Perkin-Elmer, Norwalk, CT). The initial DNA denaturation was performed at 95°C for 5 min, followed by 35 cycles of 94°C denaturation for 10 1 min, 55°C annealing for 1 min and 72°C extension of 4 min, with a final extension of 72°C for 8 min.

10 probes and Oligonucleotides sequences: The KAI1 probe (nucleotides 64-1094 of the KAI1 cDNA) used in Southern and Northern blot analyses was generated by PCR with 15 primers shown as SEQ ID NO: 13 AGTCCTCCCTGCTGCTGTGTG and SEQ ID NO:14 TCAGTCAGGGTGGGCAAGAGG. Human and rat β -actin probes were PCR products generated by templates and primers purchased from Clontech (Palo Alto, CA). The 20 primer sequences for human β -actin are shown as SEQ ID NO:15 GAGGAGCACCCCGTGCTGCTGA and SEQ ID NO:16 CTAGA AGCATTGCGGTGGACGATGGAGGGGCC and the primer sequences for rat β -actin are shown as SEQ ID NO: 17 TTGTAACTGGGACGATATGG and SEQ ID NO:18 GTCTTGATCTTCATGGTGCTAGG.

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Example 1

Cloning of the KAI1 Gene

To clone the gene on human chromosome 11 responsible for the metastasis suppression of AT6.1 30 prostatic cancer cells, genomic DNA fragments from the p11.2-13 region were isolated using human-specific Alu element-mediated PCR (Alu-PCR) (Nelson, D.L. et al Proc. Natl. Acad. Sci. U.S.A., 86:6686-6690) with DNAs from the metastasis suppressed microcell hybrid AT6.1-11-1* and the 35 non-suppressed hybrids AT6.1-11-2 and AT6.1-11-3. The

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- Alu-PCR fragments found only in the AT6.1-11-1 DNA were then used as probes to screen a cDNA library prepared from the suppressed cell hybrid clone AT6.1-11-1* that contains human chromosomal region 11cen-p13. Of five cDNA clones obtained, all were expressed in the suppressed hybrid but not in the nonsuppressed hybrids as detected by reverse transcription-polymerase chain reaction (RT-PCR) using primers derived from these cDNA sequences. Northern analysis of RNA isolated from human prostate and cell lines AT6.1, AT6.1-11-1*, -2 and -3 revealed that two of the cDNA clones detected a 2.4 hb and 4.0 kb transcript respectively in human tissue and the suppressed AT6.1-11-1* cells. The results of a Northern blot for one such clone, designated KAI1 for Kang Ai (Chinese for anti-cancer), are shown in Figure 1 and clearly demonstrate that KAI1 mRNA was abundant in the metastatic suppressed AT6.1-11-1* cells but absent from the parental AT6.1 cells and the nonsuppressed hybrids. Therefore, the KAI1 clone was analyzed further.

To confirm that the KAI1 gene was isolated from the p11.2-13 region of human chromosome 11 involved in metastasis suppression, Southern blot analysis was conducted on 15 µg of genomic DNA from human placenta, rodent cells (A9 and AT6.1) and human-rodent microcell hybrids (AT6.1-11-1*, AT6.1-11-2 and AT6.1-11-3), digested with Hind III, separated on a 1.2% agarose gel and hybridized with KAI1 probe. The results shown in Figure 2 demonstrate that only the cell hybrids that have the p11.2-13 region involved in metastasis suppression (AT6.1-11-1*) have the pattern observed with normal human DNA when hybridized to KAI1 probe. Fluorescence *in situ* hybridization of a KAI1 probe to metaphase chromosomes further localized KAI1 to the p11.2 region.

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Example 2

Nucleotide And Deduced Amino Acid Sequences of
the KAI1 cDNA

The nucleotide and deduced amino acid sequences of the KAI1 cDNA are shown in Figure 3. The KAI1 cDNA has a single open reading frame from nucleotide positions 166 to 966, predicting a protein of 267 amino acids with a calculated molecular weight of 29,610 daltons. An Alu element was present in the 3'-untranslated region of the cDNA. The predicted protein had four hydrophobic and presumably transmembrane domains and one large extracellular hydrophilic domain with three potential N-glycosylation sites. As noted earlier, the KAI1 cDNA sequence is identical to three cDNA clones from human lymphocytes, C33, R2 and IA4.

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Example 3

Determination That KAI1 Is A Metastasis Suppressor Gene

To investigate if KAI1 is the gene responsible for metastasis suppression in AT6.1-11-1*, KAI1 cDNA was subcloned into a constitutive expression vector and transfected into parental AT6.1 cells as follows. In brief, KAI1 cDNA was cloned into pCMVneo, in which transcription is driven by the constitutive human cytomegalovirus promoter (Eliyahu, D. et al Proc Natl Acad Sci U.S.A., (1989) 86:8763-8767). The resultant plasmid pCMV-KAI1 was transfected into AT6.1 cells by calcium phosphate precipitate method and the vector alone was also transfected as a negative control. Individual transfectants were isolated in selection medium (RPMI-1640 plus 10% fetal calf serum, 2units/ml pen-strep and 500 ug/ml neomycin). Exponentially growing untransfected AT6.1, AT6.1-11-1* and AT6.1-11-2 cells and exponentially growing vector (AT6.1VEC-1, AT6.1VEC-2 and AT6.1VEC-3) and KAI1 (AT6.1KAI-1, AT6.1KAI-2 and AT6.1KAI-3) transfectants

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o were collected by scraping and cell clumps were broken up by gentle pipetting. The cell suspension was placed in a tube and allowed to stand at room temperature for 30 min. Cells from the supernatant suspension were collected, washed, and resuspended in cold PBS at 10^6 cells/ml.

5 Four-to-five-week-old male Ncr nu/nu nude mice (National Cancer Institute Animal Program, Bethesda, MD) were injected with 0.1 ml of the indicated cell suspension (10^5 cells) (the column designated "Clone" in Table 1) subcutaneously at sites on both the right and left

10 midlateral, about 1/4 of the distance from the base of the skull to the base of the tail. About 6 weeks after injection, the tumors were weighed and the lungs were inflated with Bouin's solution. Tumor foci on the surface of lungs were scored under a dissecting microscope.

15 Individual transfectants were analyzed for *KAI1* expression and for their ability to suppress lung metastases and the results of one experiment are shown in Table 1.

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TABLE 1

Clones	KAI1* mRNA Level	Latency ⁺ (days)	Tumor Age (Days)	Tumor Weight(g) @ Excision	Number of Mice with Metastases	Mean Number ⁺ Metastasis Per Mouse Mean (#mice)	P
5	AT6.1	0	4.3	27	2.58	19/19	58(32-135)
	AT6.1-11-1*	10	3.7	37	2.79	6/7	7(0-9)
	AT6.1-11-1-2	0	4.2	37	2.78	6/6	26(20-40)
10	AT6.1VEC-1	0	4.9	43	2.32	17/17	30(16-57)
	AT6.1VEC-2	0	4.0	43	3.26	17/17	30(12-71)
	AT6.1VEC-3	0	5.5	43	2.57	18/18	47(15-183)
15	AT6.1KAI-1	10	4.2	43	3.99	18/20	<0.001 [†]
	AT6.1KAI-2	7	4.5	41	1.79	17/19	<0.001 [†]
	AT6.1KAI-3	1	4.5	43	2.56	18/19	<0.02 [†]

The data shown in this table are from a large, age-matched cohort of "side-by-side" nude mice, with cells inoculated at the same time.

20 * KAI1 expression was determined by Northern blot analysis. The KAI1 signals on the Northern blot were scored by a densitometer. The value for AT6.1KAI-1 was standardized to 10 and the values for other clones were adjusted accordingly.

 + Latency is the time following injection for a palpable tumor to appear.

 + The numbers in parentheses indicate the range of metastases in individual mice.

 † Compared to the number of metastases with AT6.1-11-2 cells.

25 † Compared to the mean number of metastases with all of the three vector transfectants.

The results presented show that expression of KAI1 resulted in a significant suppression of the number of lung metastases per mouse but did not affect the growth rate of the primary tumor. Further, whereas the parental AT6.1 cells yielded 58 metastasis per mouse when injected subcutaneously into nude mice, two transfectants with levels of KAI1 mRNA expression similar to the high level of expression observed in AT6.1-11-1* cells gave only 6 or 7 lung metastases per animal. In contrast, the three

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vector control transfectants produced 30-47 lung metastases per mouse, which is on average 5.5 times the number of metastases observed with the 2 KAI1 transfectants with high KAI1 mRNA expression (AT6.1KAI-1 and AT6.1KAI-2). In addition, while the AT6.1KAI-3 clone 5 which had low KAI1 expression produced 23 lung metastases, this was still significantly less than the mean number of lung metastases for control transfectants. Finally, Northern analysis showed that KAI1 expression was undetectable or very low in 28 lung metastases from KAI1 transfectants suggesting that selection for cells with 10 absent or reduced KAI1 expression resulted in metastasis formation. These results indicate that the metastatic ability of AT6.1 cells is suppressed by KAI1 expression.

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Example 4

KAI1 mRNA Expression In Cell Lines Derived From Metastatic Human Prostate Tumors

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To determine whether KAI1 mRNA expression was reduced in human metastatic prostate tumors relative to expression in normal human prostate, 15 µg total RNA from human normal prostate tissue and from cell lines derived 25 from metastatic prostate cancers (Kaighn, M.E. et al (1979) Invest. Urol. :17:16; Horoszewicz, J.J. et al. in Models for Prostate Cancer, G.P. Murphy. Ed. (Alan R. Liss, Inc., New York, 1980). pp 115-132: T. Iizumi. et al. (1987) J. Urol. 137:1304, D.D. Mickey et al., in Models for Prostate Cancer. G. P. Murphy. Ed. (Alan R. Liss, Inc. New York, 1980), pp. 67-84) were denatured with formamide, electrophoresed fractionated on a 1.2% agarose 30 gel and hybridized sequentially to KAI1 and human β-actin probes. The results of this Northern blot analysis are shown in Figure 4 and clearly demonstrate that KAI1 expression was significantly reduced in the human cell lines derived from metastatic prostate tumors (PC-3, LNCaP, TSU-Pr1 and DU145) when compared to normal prostate

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• (prostate). In addition, while longer exposures (4 days at -80 C) of the autoradiogram shown in Figure 4 (overnite at -80 C) revealed expression of KAI1 mRNA in all of the tumor cells, the level of expression was still much lower than in normal prostate.

5 To rule out the possibility that the metastasis suppression by KAI1 was due to an indirect immune mechanism, two other experiments were performed. First, parental AT6.1 cells, cell hybrid clone AT6.1-11-1*, or a KAI1 transfectant (AT6.1 KAI-1) were inoculated into the 10 leg of severe combined immune deficient (SCID) mice at 5×10^5 cells/mouse. When tumors reached 3-5 cm³, the leg with tumor was surgically removed and animals were followed until 50 to 60 days post inoculation. Lung metastases for each mouse were analyzed as described for Table 1. For 15 AT6.1, 9/9 mice had lung metastases with an average number of 83 per mouse. For AT6.1-11-1*, 4/9 mice had lung metastases with an average number of 6 per mouse. For AT6.1KAI-1, 2/7 mice had lung metastases with an average 20 number of 2 per mouse. These studies demonstrated that even in SCID mice, which are more immune compromised than nude mice, metastasis suppression was observed.

Second, highly metastatic rat mammary cancer cells into which the KAI1 gene was introduced via microcell-mediated chromosome transfer, retained their 25 ability to metastasize (Rinker-Schaefer, C.W. et al. (1994) Cancer Res., 54:6249-6256) even though the hybrids expressed similar level of KAI1 mRNA. Based upon these data, a more direct mechanism appears to be responsible for the metastasis suppression by KAI1. Consistent with 30 this possibility, high KAI1 expressing AT6.1-11-1* hybrid cells have about 50% reduction in their invasive ability as compared to parental AT6.1 cells or nonsuppressed AT6.1-11-2 hybrid cells in Boyden chamber assay. In brief, Boyden chamber invasion assays were performed as 35 described by J. Vukanovic et al. (1993) Cancer Res., 53:

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• 1833), using matrigel coated filters and 5% fetal bovine serum as chemoattractant in the lower well. During the 12 hours of the assay, 19 ± 3 parental AT6.1 cells per high power field invaded through the matrigel filters versus 10 ± 2 for the metastasis suppressed AT6.1-11-1* hybrid cells and 18 ± 2 for the nonsuppressed AT6.1-11-2 hybrid cells.

Example 5

Expression of KAI1 Gene In Human Tissues

10 To evaluate the expression level of KAI1 gene in various human tissues, Northern analysis was performed on RNA isolated from multiple human tissues. In brief, a human multiple tissue Northern blot purchased from Clontech (Palo Alto, CA) was hybridized sequentially with KAI1 and human β -actin probes under conditions described 15 in the Methods section. The results presented in Figure 5 show that the 2.4 kb KAI1 transcript was detected in all the human tissues tested, with high abundance in prostate, lung, liver, kidney, bone marrow and placenta; moderate abundance in mammary gland, pancreas, skeletal muscle and 20 thymus; and low expression in brain, heart, ovary, stomach and uterus.

Example 6

Conservation Of The KAI1 Gene Across Species

25 To determine if the KAI1 gene is evolutionarily conserved across species, a zoo blot containing EcoRI-digested genomic DNA from various species was purchased from Clontech (Palo Alto, CA) and hybridized with KAI1 probe. The results presented in Figure 6 show that the 30 evolutionary conservation of KAI1 coding sequence is high in human, monkey, dog and rabbit and moderate in cow, rat, mouse. The evolutionary conservation and wide tissue distribution for KAI1 suggest that the gene may have an 35 essential biological function.

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• Example 7

Correlation Of Altered KAI1 Expression
In Human Tissue Samples With Metastasis

5 Tumor biopsies of liver metastases from prostate cancer patients and liver biopsies from healthy patients are analyzed for KAI1 mRNA expression by Northern blotting. KAI1 mRNA expression is lost in the tumor samples indicating that the presence of liver metastases in the prostate cancer patients is correlated with altered KAI1 expression.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: THE GOVERNMENT OF THE UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES AND JOHN HOPKINS UNIVERSITY

(ii) TITLE OF INVENTION: DIAGNOSTIC METHODS AND GENE THERAPY USING REAGENTS DERIVED FROM THE HUMAN METASTASIS SUPPRESSOR GENE KAI1

(iii) NUMBER OF SEQUENCES: 18

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(v) COMPUTER READABLE FORM:
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(B) COMPUTER: IBM PC COMPATIBLE
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WORDPERFECT 5.1

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- 32 -

° (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGAAGATCAA GTTGAAGAGG

20

(2) INFORMATION FOR SEQ ID NO:2:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

15 GGGACCTCAT TTCCTAGCTG

20

(2) INFORMATION FOR SEQ ID NO:3:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGAAACTGC TCTTGTGCG

19

25 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCAGCTCTTG GCTCCCCATT

20

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o (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TGGGCACGGG TTTCAGGAAA T

21

10 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TGCAGAGAGC CCCAAATGCA

20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

25 AGGGTGAGCC GTGAGGCACAA

20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

20 TGCTGAGAGT ACCCAGATGC

20

- 34 -

• (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GATGGCCACA CCCACGCC

19

10 (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGCATGGAGA AGGTGCAGGC

20

20 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCTCTTGCCC ACCCTGACTGA

21

20 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TTCACACCAT TCTCCTGCCT

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(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGTCCTCCCT GCTGCTGTGT G

21

(2) INFORMATION FOR SEQ ID NO:14:

10

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCAGTCAGGG TGGGCAAGAG G

21

(2) INFORMATION FOR SEQ ID NO:15:

20

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAGGAGCACC CCGTGCTGCT GA

22

(2) INFORMATION FOR SEQ ID NO:16:

30

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTAGAACGAT TTGCGGTGGA CGATGGAGGG GCC

33

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o
(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TTGTAACCAA CTGGGACGAT ATGG

24

10 (2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTCTTGATCT TCATGGTGCT AGG

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Claims

1. A method for detecting the presence of malignant cancer in a subject comprising: comparing *KAI1* gene sequence, *KAI1* mRNA or *KAI1* protein of said subject to wild-type *KAI1* gene sequence, mRNA or protein, an observed alteration in *KAI1* gene sequence, *KAI1* mRNA, or *KAI1* protein of said subject as compared to wild-type indicating the presence of malignant cancer in said subject.

10 2. The method of claim 1, wherein *KAI1* gene sequences are compared.

15 3. The method of Claim 2 wherein said alteration in *KAI1* gene sequence is detected by Southern hybridization.

20 4. The method of Claim 2, wherein said alteration in *KAI1* gene sequence is detected by cloning *KAI1* genes of said subject and sequencing all or part of the cloned gene.

5. The method of claim 2, wherein said alteration in *KAI1* gene sequence is detected by PCR-SSCP.

25 6. The method of claim 5, wherein the primers used in said PCR step are derived from *KAI1* cDNA.

7. The method of claim 5, wherein the primers used in said PCR-SSCP are selected from SEQ ID NOS:1-12.

30 8. The method of claim 1 wherein *KAI1* mRNA molecules are compared.

35 9. The method of claim 8, wherein said alteration in *KAI1* mRNA is detected by Northern blotting.

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10. The method of claim 8, wherein said alteration in KAI1 mRNA is detected by RT-PCR.

11. The method of claim 10, wherein the primers used in said PCR step are derived from KAI1 cDNA.

5 12. The method of claim 8, wherein said alteration in KAI1 mRNA is detected by RT-PCR-SSCP.

10 13. The method of claim 12, wherein the primers used in said PCR step are derived from KAI1 cDNA.

14. The method of claim 1, wherein KAI1 proteins are compared.

15 15. The method of claim 14, wherein said alteration in KAI1 protein is detected by Western blotting.

20 16. The method of claim 15, wherein said alteration in KAI1 protein is detected by immunohistochemistry.

17. Antibodies having specific binding affinity for KAI1 protein or peptide fragments thereof.

25 18. The antibodies of claim 17, wherein said antibodies are monoclonal antibodies.

30 19. Purified and isolated primers having nucleic acid sequences selected from the group consisting of SEQ ID NOS: 1-12.

35 20. A diagnostic kit for use in detecting the presence of malignant cancer in a subject, said kit comprising:

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primers having nucleic acid sequences selected from the group consisting of SEQ ID NOs: 1-12.

21. A gene therapy method for a subject having altered *KAI1* expression comprising administering to said subject a recombinant expression vector having a nucleic acid sequence capable of directing host organism synthesis of wild-type *KAI1* protein.

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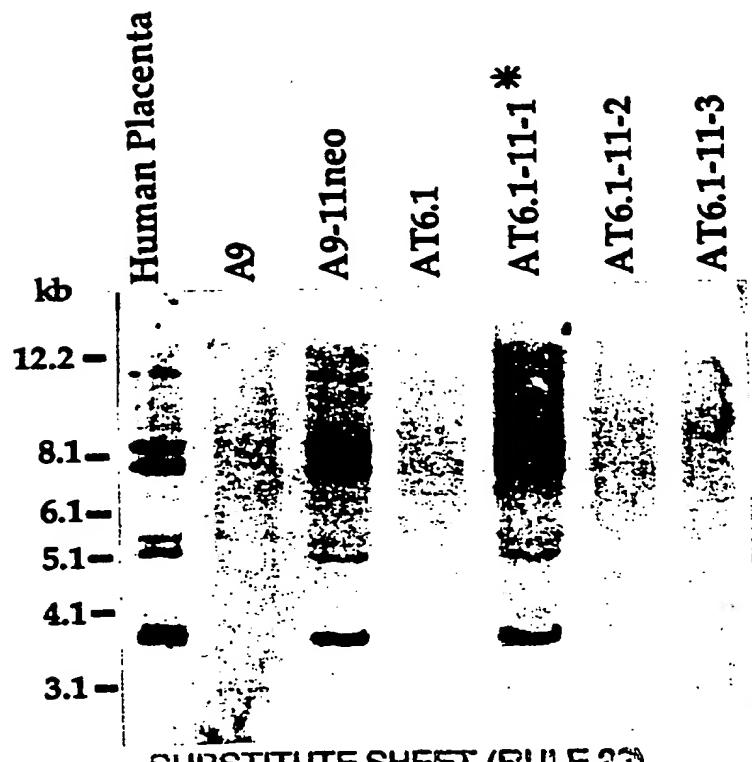
35

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FIG. 1



FIG. 2



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FIG. 3

1 CCGACTGAGGCACGAGCGGGTGACGCTGGGCCTGCAGCGCGGAGCAGAAAGCAGAACCCG
 61 CAGAGTCCTCCCTGCTGCTGTGGACACACGTGGGCACAGGCAGAAGTGGGCCCTGTG
 121 ACCAGCTGCACTGGTTCTGGAAGGAAGCTCCAGGACTGGCGGGATGGGCTAGCCTGT
 1 M G S A C
 181 ATCAAAGTCACCAAATACTTTCTCTTCAACTTGATCTCTTATCCTGGCGCA
 6 I K V T K Y F L F L F N L I F F I L G A
 241 GTGATCCTGGGCTTCGGGCTGGATCCTGGCCGACAAGAGCAGTTCATCTCTGTGCTG
 26 V I L G F G V W I L A D K S S F I S V L
 301 CAAACCTCCCTCAGCTCGCTTAGGATGGGGCCTATGCTTCATCGGCGTGGGGCAGTC
 46 Q T S S S S L R M G A Y V F I G V G A V
 361 ACTATGCTCATGGGCTTCTGGGCTGCATGGCGCCGTCACGAGGTCCGCTGCCTGCTG
 66 T M L M G F L G C I G A V N E V R C L L
 421 GGGCTGTACTTTGCTTCCTGCTCCTGATCCTCATGGCCAGGTGACGGCCGGGCCCTC
 86 G L Y F A F L L I L I A Q V T A G A L
 481 TTCTACTTCAACATGGCAAGCTGAAGCAGGAGATGGCCGCATCGTACTGAGCTCATT
 106 F Y F N M G K L K Q E M G G I V T E L I
 541 CGAGACTACAACAGCAGTCGCAGGACAGCCTGCAGGATGCCTGGACTACGTGCAGGCT
 126 R D Y N S S R E D S L Q D A W D Y V Q A
 601 CAGGTGAAGTGTGCGGCTGGTCAGCTTACAACTGGACAGACAACGCTGAGCTCATG
 146 Q V K C C G W V S F Y N W T D N A E L M
 661 AATCGCCCTGAGGTACACCTACCCCTGTTCTGCGAAGTCAAGGGGAAAGAGGACAACAGC
 166 N R P E V T Y P C S C E V K G E E D N S
 721 CTTCTGTGAGGAAGGGCTCTGCGAGGCCCGGCAACAGGACCCAGAGTGGCAACCAC
 186 L S V R K G F C E A P G N R T Q S G N H
 781 CCTGAGGACTGGCCTGTGTACCAAGGAGGGCTGCATGGAGAAGGTGCAGGGTGGCTGCAG
 206 P E D W P V Y Q E G C M E K V Q A W L Q
 841 GAGAACCTGGCATCATCCTCGCGTGGCGTGGGTGTGGCCATCATCGAGCTCCTGGGG
 226 E N L G I I L G V G V G V A I I E L L G
 901 ATGGTCTGTCCATCTGCTGTGCCACGTCCATTCCGAAGACTACAGCAAGGTCCCC
 246 M V L S I C L C R H V H S E D Y S K V P
 961 AAGTACTGAGGCAGCTGCTATCCCCATCTCCCTGCCTGGCCCCAACCTCAGGGCTCCA
 266 K Y *
 1021 GGGGTCTCCCTGGCTCCCTCCAGGCCTGCCTCCACTTCACATGCAGAACCCCTTTG
 1081 CCCACCTGACTGAAAGTAGGGGGCTTCTGGGCCTAGCGATCTCTCCCTGGCCTATCCG
 1141 CTGCCAGCCTTGAGCCCTGGCTGTTCTGCTGGTCTGCTCACCGCCCATCAGGGTTCT
 1201 CTTATCAACTCAGAGAAAATGCTCCCCACAGCGTCCCTGGCGCAGGTGGCTGGACTTC
 1261 TACCTGCCCTCAAGGGTGTATATTGTATAGGGCAACTGTATGAAAAATTGGGGAGGA
 1321 GGGGGCCGGCGCGGTGCTCACGCCGTAAATCCCAGCAGTGTGTTGGGAGGGCGAGGGGGTG
 1381 GATCACGAGGTCAAGGAGATCGAGACCATCCTGGCTAACATGGTAAACCCCCGTCTACT
 1441 AAAAATACAAAAAAATTAGCCGGCGCGGTGGCGGGCACCTGTAGTCCCAGCTACTTG
 1501 GGAGGGCTGAGGCAGGAGAATGGTGTGAACCCGGAGCGGGAGGTTGCAGTGAGCTGAGATC
 1561 GTGCTACTGCACTCCAGCCTGGGGACAGAAAGAGACTCCGTCTCAAAAAAAA
 1621 AAAA

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FIG. 4

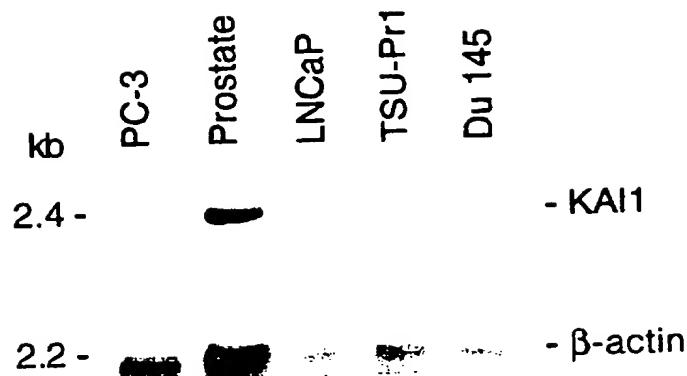
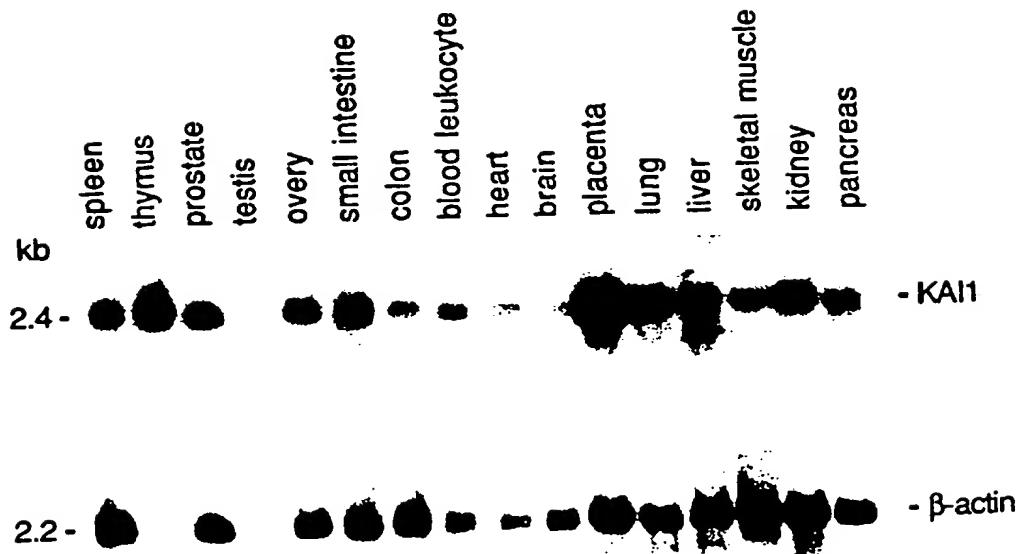
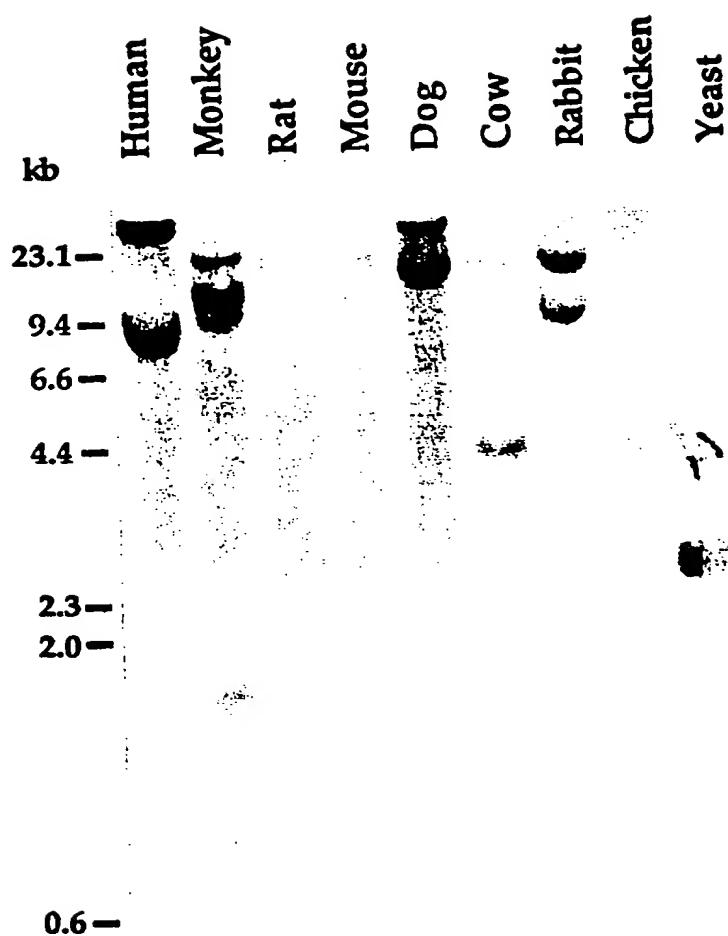


FIG. 5



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FIG. 6



INTERNATIONAL SEARCH REPORT

Inv. No. International Application No.
PCT/US 96/05848A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 C07K14/725 C07K16/30 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>EIGHTY-SIXTH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, TORONTO, ONTARIO, CANADA, MARCH 18-22, 1995. PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING 36 (0). 1995. 105. ISSN: 0197-016X, XP002011239</p> <p>DONG J-T ET AL: "A metastasis suppressor gene KAI1 for prostate cancer from the p11.2 region of human chromosome 11." see abstract</p> <p>---</p> <p>-/-</p>	1-21



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *'A' document defining the general state of the art which is not considered to be of particular relevance
- *'E' earlier document but published on or after the international filing date
- *'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *'O' document referring to an oral disclosure, use, exhibition or other means
- *'P' document published prior to the international filing date but later than the priority date claimed

*'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

*'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

*'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

*'A' document member of the same patent family

1

Date of the actual completion of the international search

21 August 1996

Date of mailing of the international search report

03.09.96

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Authorized officer

Molina Galan, E

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/05848

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		Relevant to claim No.
Category	Citation of document, with indication, where appropriate, of the relevant passages	
X	J. IMMUNOL. 149:2879-2886(1992). XP002011240 IMAI ET AL.: "C33 antigen recognized by monoclonal antibodies inhibitory to human T cell leukemia virus type 1-induced syncytium formation is a member of a new family of transmembrane proteins including CD9, CD37, CD53, and CD63" cited in the application see the whole document ---	17,18
X	JOURNAL OF IMMUNOLOGY, vol. 148, 1992, BALTIMORE US, pages 2826-2833, XP002011241 GIL ET AL.: "A member of the tetra spans transmembrane protein superfamily is recognised by a monoclonal antibody raised against an HLA class I-deficient, lymphokine-activated killer-susceptible, B lymphocyte line" cited in the application see the whole document ---	17,18
A	CANCER RESEARCH, vol. 51, 1991, MD US, pages 3788-3792, XP000579619 ICHIKAWA ET AL.: "Genetic factors and suppression of metastatic ability of prostatic cancer" cited in the application see the whole document ---	1-21
A	CANCER RESEARCH, vol. 52, 1992, MD US, pages 3486-3490, XP002011242 ICHIKAWA ET AL.: "Localisation of metastasis suppressor gene(s) for prostatic cancer to the short arm of human chromosome 11" cited in the application see the whole document ---	1-21
A	85TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, SAN FRANCISCO, CALIFORNIA, USA, APRIL 10-13, 1994. PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING 35 (O). 1994. 186. ISSN: 0197-016X, XP002011243 DONG J-T ET AL: "Cloning and characterization of a putative metastasis suppressor gene on human chromosome 11p11.2-13 for prostatic cancer." see abstract ---	1-21
1		-/-

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/05848

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CANCER RESEARCH, vol. 54, 1994, MD US, pages 6249-6256, XP002011244 RINKER ET AL.: "Differential suppression of mammary and prostate cancer metastasis by human chromosomes 17 and 11" cited in the application see the whole document ---	1-21
P,X	SCIENCE, (1995 MAY 12) 268 (5212) 884-6., XP002011245 DONG ET AL.: "KAI1, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2" see the whole document ---	1-21
P,X	CANCER RESEARCH, vol. 56, 15 April 1996, MD US, pages 1751-1755, XP002011246 ADACHI ET AL.: "Correlation of KAI1/CD82 gene expression with good prognosis in patients with non-small cell lung cancer. " see the whole document -----	1-21

INTERNATIONAL SEARCH REPORT

II. International application No.

PCT/US 96/05848

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Please see Further Information sheet enclosed.
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark : Although claim 21 is directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.